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Studies on Graft Copolymerization of 2-Acrylamido-2-methylpropanesulfonic Acid onto Protein Initiated by Ammonium Persulfate

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The protein, collagen, has been chemically modified by graft copolymerization of 2-acrylamido-2-methylpropanesulfonic acid (AMPS) in an aqueous medium using ammonium persulfate as an initiator under argon atmosphere. A plausible reaction mechanism of grafting has been suggested. Evidence of grafting was obtained by comparison of FTIR spectra of collagen and homopolymer-free collagen-*g*-poly(2-acrylamido-2-methylpropanesulfonic acid) as well as solubility characteristics and gravimetric analysis of the products. Evidence of grafting was obtained by comparing FTIR, thermogravimetric curves of collagen and the graft copolymer as well as solubility characteristics of the products. The synthetic conditions were systematically optimized through studying the influential factors including temperature, concentration of the initiator, 2-acrylamido-2-methylpropanesulfonic acid monomer and collagen substrate.

Key Words: Graft copolymer, Collagen, 2-Acrylamido-2-methylpropanesulfonic acid.

INTRODUCTION

Graft copolymerization of hydrophilic vinyl monomers is a well-known technique employed by polymer chemists for significantly modifying the chemical and physical properties of the synthetic or natural starting materials with minimum degradation of the original properties[1-5]. Graft copolymers are prepared by first generating free radicals on the polysaccharide backbone and then allowing these radicals to serve as macroinitiators for the vinyl monomers. These biodegradable and low cost graft copolymers, with new properties can be used in many applications such as textiles, paper industry, agriculture, medical treatment, in petroleum industry as flocculants and thickening agents[2,3] and also development of selective permeable membranes[6], sorption agents[7] and in fabrication of drug delivery systems[8,9].

Proteins are widely distributed in nature and are synthesized mainly in animals, *i.e.* collagen, keratin, gelatin, *etc*. and in a few plants such as Soya. In general, proteins are high molecular weight polymers and their solubility in aqueous solutions is difficult. Two efficient methods for preparation of aqueous soluble proteins are alkaline and enzymatic hydrolysis. According to the literature survey, a few studies have been reported in the case of graft copolymer based on protein[10-12].

Though much work has been reported on the grafting of 2-acrylamido-2-methylpropanesulfonic acid onto various polysaccharides.Therefore, The present investigation deals with the detailed study of some major factors which affect graft copolymerization of 2-acrylamido-2-methylpropanesulfonic acid (AMPS) onto collagen, initiated by ammonium persulfate (APS) in aqueous medium with a view to elucidate the grafting mechanism.

EXPERIMENTAL

Hydrolyzed collagen (Parvar Novin-E Tehran Co.) was industrial grade which is available in market and has nearly 25 % insoluble phosphate salt. 2-Acrylamido-2-methylpropanesulfonic acid (Merck, Darmstadt, Germany) and ammonium persulfate (Fluka, Buchs, Switzerland) were of analytical grade and used without further purification.

Graft copolymerization procedure: A pre-weighed amount of hydrolyzed collagen (0.5-2.5 g) was dissolved in 50 mL degassed distilled water and filtered to remove its insoluble salt. The solution was added to a 100 mL three-neck reactor equipped with a mechanical stirrer (RZR 2021, a three-blade propeller type, Heidolph, Schwabach, Germany) and the reactor was immersed in a thermostated water bath preset at a desired temperature (35-65 ºC). Then, a given amount of monomer, AMPS (1-5 g), was added to the flask and the mixture was stirred for 10 min. Then the initiator solution (0.01-0.05 g APS in 5 mL H_2O) was added to the mixture and continuously stirred for certain times (30-120 min). An inert gas (argon) was gently bubbled into the reactor to remove the

oxygen during the graft copolymerization reaction. The product was then worked up with methanol (200 mL) and dried in oven at 50 ºC for 5 h.

Homopolymer extraction: The graft copolymer, collagen-*g*-poly(AMPS), was freed from poly(AMPS) homopolymer, by pouring 0.30 g of the product in 50 mL of dimethyl formamide solution. The mixture was stirred gently at room temperature for 48 h. After complete removal of the homopolymer by filtration of the collagen-*g*-poly(AMPS), copolymer, the product was washed with methanol and dried in oven at 50 ºC to reach a constant weight[13].

The infrared spectra were taken on an ABB Bomem MB-100 FTIR spectrophotometer. Thermogravimetric analyses were performed on a Universal V4.1D TA Instruments (SDT Q600) with 8-10 mg samples on a platinum pan under nitrogen atmosphere. Experiments were performed at a heating rate of 10 ºC/min until 600 ºC.

Grafting parameters: The grafting parameters, *i.e.* grafting ratio (Gr %), add-on value (Ad %) and homopolymer content (Hp %), used to characterize the nature of the copolymer are defined and calculated using the following equations[14-17]:

$$Gr\ \% = 100\ (W_2 - W_0)/W_0 \quad (1)$$

$$Ad\ \% = 100\ (W_2 - W_0)/W_2 \quad (2)$$

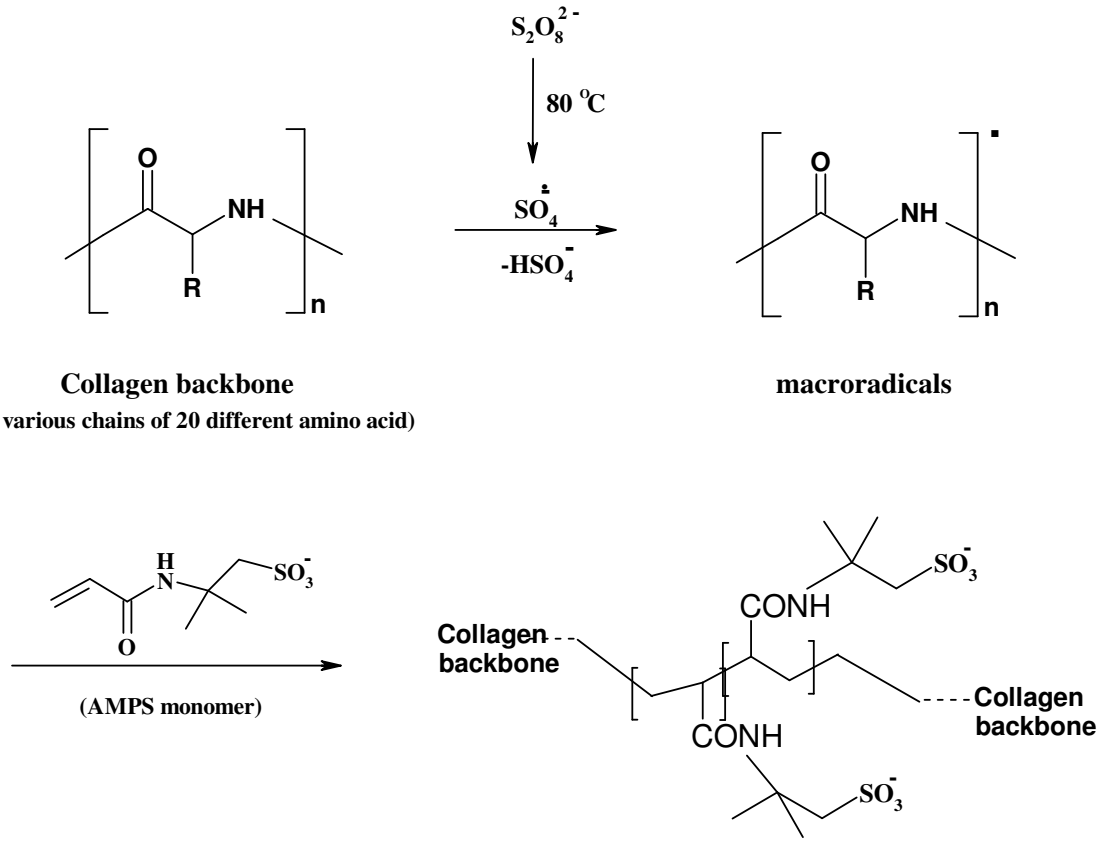
$$Hp\ \% = 100\ (W_1 - W_2)/W_1 \quad (3)$$

where W_0, W_1 and W_2 are the weight of the initial substrate, total product (copolymer and homopolymer) and pure graft copolymer (after DMF extraction), respectively.

RESULTS AND DISCUSSION

Grafting mechanism: A general reaction mechanism for graft copolymerization of AMPS onto collagen backbones in the presence of APS is shown in **Scheme-I**. In the first step, the thermally dissociating initiator, *i.e.* APS, is decomposed under heating (65 ºC) to produce sulfate anion-radicals. Then, the anion-radicals abstract hydrogen from one of the functional groups (*i.e.* COOH, SH, OH and NH_2) in side chains of the collagen backbones to form corresponding macro-initiators. These macroradicals initiate grafting of AMPS onto collagen backbones leading to a graft copolymer.

FTIR spectroscopy was used for identification of the graft copolymer. Fig. 1 shows the IR spectra of the collagen and the resulted graft copolymer. The band observed at 1658 cm^{-1} can be attributed to C=O stretching in carboxamide functional groups of substrate backbone (Fig. 1a). The broad band at 3600-3200 cm^{-1} is due to stretching of -OH groups of the collagen. The collagen-*g*-AMPS hydrogel comprises a collagen backbone with side chains that carry sulfate groups that are evidenced by a new characteristic absorption band at 1221 cm^{-1} (Fig. 1b). This peak attributed to ester sulfate stretching of AMPS. The stretching band of -NH overlapped with the OH stretching band of the collagen portion of the copolymer[18].



Scheme-I: Proposed mechanistic pathway for synthesis of collagen-*g*-poly(AMPS) coplymer

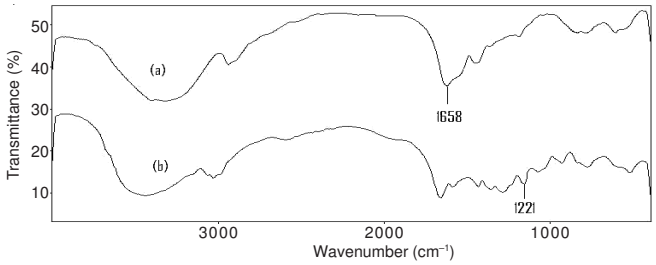


Fig. 1. FTIR spectra of collagen (a) and collagen-*g*-poly(AMPS) hydrogel (b)

Thermogravimetric analysis (TGA) was employed to thermally characterize the graft copolymer in comparison with the intact collagen (Fig. 2). The thermal stability of the grafted collagen is improved as is obvious from the TGA curve. TGA of collagen (Fig. 2a) shows a weight loss in two distinct stages. The first stage ranges between 15 and 90 ºC and shows *ca.* 12 % loss in weight. This may correspond to the loss of adsorbed and bound water[18]. No such inflexion was observed in the TGA curve of collagen-*g*-AMPS. This indicated that the grafted copolymers were resistant to moisture absorption. The second stage of weight loss starts at 240 ºC and continues up to 430 ºC during which there was 68 % weight loss due to the degradation of collagen. Grafted samples, however, show almost different behaviour of weight loss between 15 and 550 ºC (Fig. 2b). The first stage of weight loss starts at 220 ºC and continues up to 340 ºC due to the degradation of collagen. The second stage from 340 to 450 ºC may contribute to the decomposition of different structure of the graft copolymer. The appearance of these stages indicates the structure of collagen chains has been changed, which might be due to the grafting of AMPS chains. In general, the copolymer had lower weight loss than collagen. This means that the grafting of collagen increases the thermal stability of collagen in some extent.

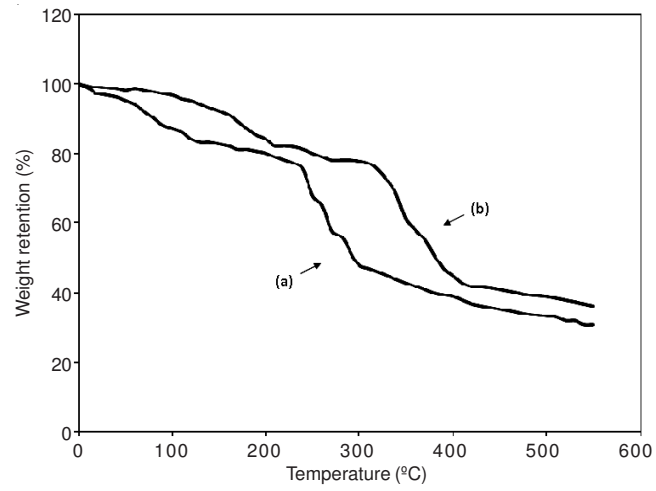


Fig. 2. TGA curves of (a) Collagen and (b) collagen-*g*-poly(AMPS)

Optimization of copolymerization reaction: Since polymerization variables determine the extent of grafting and homopolymer amount, certain factors affecting the grafting parameters were investigated to achieve the optimum condition of polymerization. Therefore, we optimized the grafting of 2-acrylamido-2-methyl propan sulfonic acid onto collagen in homogenous aqueous media by changing temperature, the initial concentration of monomer, initiator and the relative amount of the substrate. Within the range of the amount of the reactants used, our preliminary studies showed no considerable dependence between the reaction time and the grafting extent.

Effect of initiator concentration: Grafting of 2-acrylamido-2-methylpropanesulfonic acid onto collagen backbones was carried out at various initiator concentrations (0.01-0.07 mol/L), as shown in Fig. 3. It has been observed that the % grafting and % add-on increase initially on increasing the ammonium persulfate concentration up to 0.04 mol/L, but decrease with further increase in initiator concentration. The initial increase in % grafting and % add-on may be ascribed to the increase of the active sites on the backbone of the collagen arising from the attack of APS as a initiator.

Subsequent decrease in swelling is originated from an increase in terminating step reaction *via* bimolecular collision which, in turn, causes to enhance crosslinking density. In addition, the free radical degradation of collagen backbones by sulfate radical-anions is an additional reason for decrease of per cent graft copolymer at higher APS concentration. The proposed mechanism for this possibility is reported in the previous work[19].

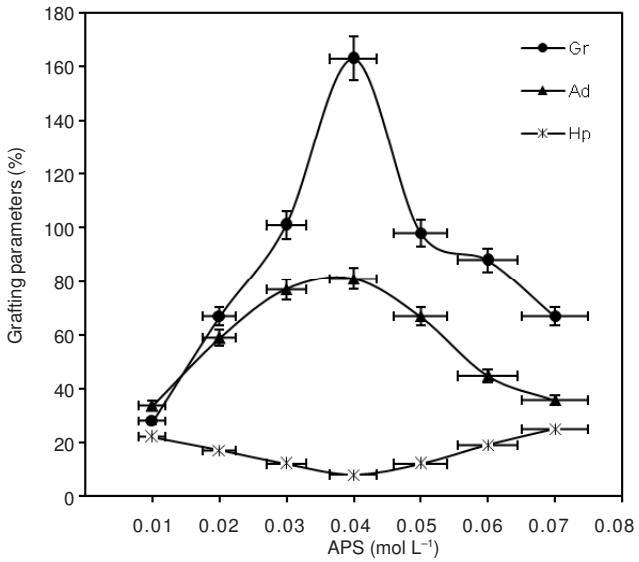


Fig. 3. Effect of initiator concentration on the grafting parameters; Reaction conditions: Collagen 2 wt %, AMPS 0.65 mol L^{-1}, temperature 55 ºC, time 80 min

Effect of monomer concentration: The AMPS concentration was varied from 0.2 to 1.4 mol/L to study its effects on grafting parameters (Fig. 4). These parameters were found to be increased by enhancement of 2-acrylamido-2-methylpropanesulfonic acid concentration from 0.2 up to 0.8 mol/L. This behaviour can be attributed to the increase of monomer concentration in the vicinity of the collagen backbone and consequent greater availability and enhancement chances for molecular collisions of the reactants. The decrease in % grafting and % add-on after a certain level of AMPS (0.8 mol/L) is probably due to preferential homopolymerization over graft copolymerization as well as increasing the viscosity of reaction

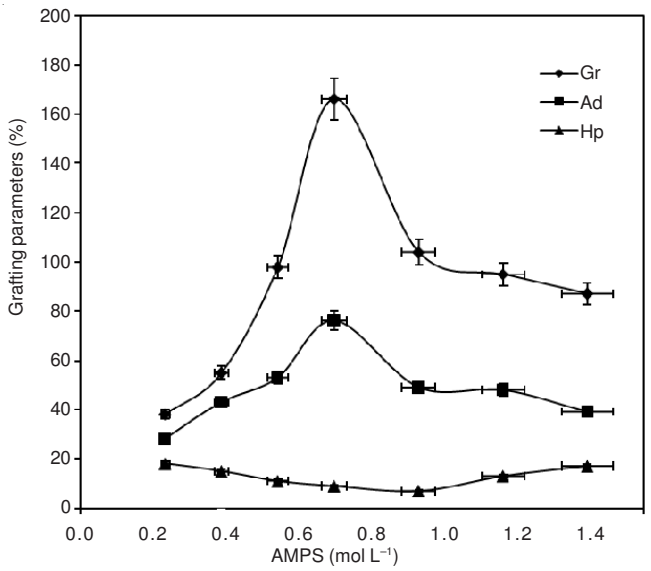


Fig. 4. Effect of the monomer concentration on the grafting parameters; Reaction conditions: Collagen 2 wt %, APS 0. 04 mol L^{-1}, temperature 55 ºC, time 80 min

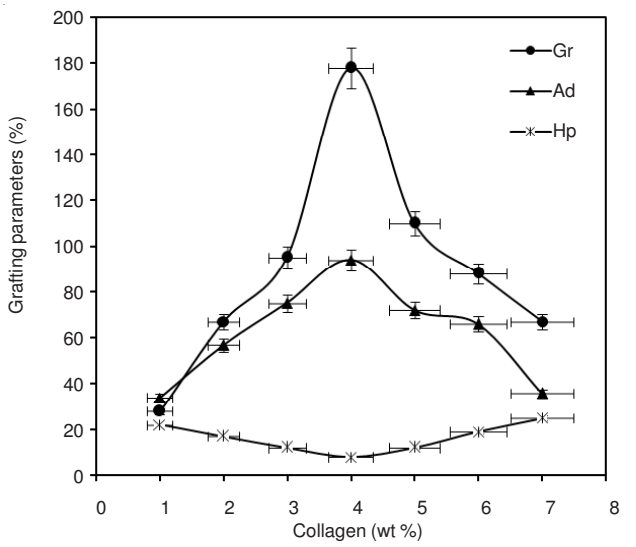


Fig. 5. Grafting parameters as functions of collagen concentration; Reaction conditions: APS 0.04 mol L^{-1}, AMPS 0.8 mol L^{-1}, temperature 55 ºC, time 80 min

medium, which hinders the movement of free radicals. Needless to say, the increase in the chain transfer to monomer molecules may be other possible reason for the diminished grafting at higher 2-acrylamido-2-methylpropanesulfonic acid concentrations. Similar observations have been reported for the grafting of ethyl acrylate onto cellulose[18,19] and methyl acrylate onto starch[20].

Effect of collagen concentration: The results obtained by changing the collagen concentration for the graft polymerization are presented in Fig. 5. It is evident from the figure that the % grafting and % add-on increase with increase in collagen concentration up to 4 wt % and then decrease with further increment of protein level. The initial increase may be due to the availability of more grafting sites, where collagen can be grafted. Subsequent decrease in grafting parameters, % grafting and % add-on, in increasing collagen content more than 4 wt %, can be explained on the basis of increase in viscosity of the medium and a decrease in the diffusion of monomers to active sites to produce graft copolymer. This observation is in close agreement with the results obtained by Zhang and Tan[21].

Effect of reaction temperature: To study the influence of the reaction bath temperature on the grafting parameters, the grafting of 2-acrylamido-2-methyl propan sulfonic acid onto collagen was carried out at seven temperatures ranging from 40 to 100 ºC. Fig. 6 exhibits the effect of polymerization temperature on the grafting parameters. Grafting percentage (% Gr) is increased with increasing the temperature from 40 to 60 ºC and then decreased. This behaviour may be related to the mobility of reactive free radical sites. Moreover, higher temperatures increase the solubility of the reactants. However, grafting was decreased as the bath temperature was raised beyond 60 ºC. This can be accounted for in terms of chain radical termination at higher temperatures. Premature termination of growing chains and instability of the APS-protein complex are presumably another reasons for reduced amount of grafting beyond 60 ºC. At higher temperatures, the rate of

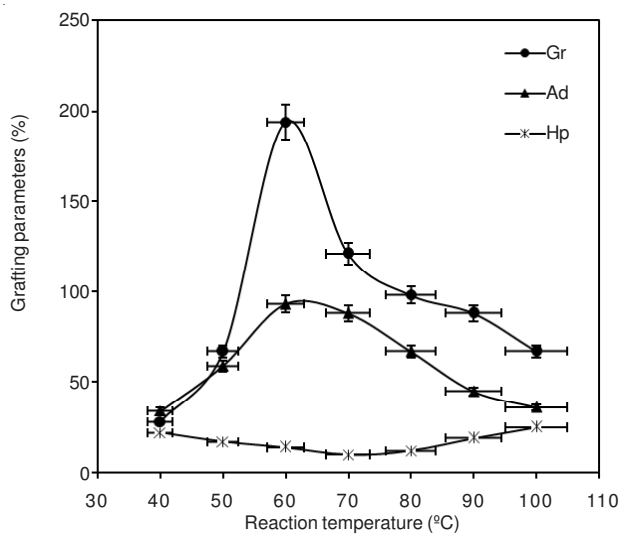


Fig. 6. Effect of the reaction temperature on the grafting parameters; Reaction conditions: Collagen 4 wt %, APS 0.04 mol L^{-1}, AMPS 0.8 mol L^{-1}, time 80 min

termination of the growing chain is increased and the monomer is volatilized out to some extent[22].

Effect of reaction time: Grafting of 2-acrylamido-2-methyl propan sulfonic acid onto collagen backbones was carried out at various polymerization times as shown in Fig. 7. The % grafting and % add-on increased with increase in the reaction time up to 90 min and thereafter, these parameters gradually decreased. It is obvious that the longer the reaction time, the better the graft copolymerization yield. The grafting loss may be attributed to decrease of all the consuming reactants. In addition, the decreased number of available active free radical sites for grafting and the retardation of diffusion of reactants, because of the long grafted chains at the kC surface, may be other possible reasons for the diminished grafting at longer reaction times. Similar time dependency of grafting parameters was reported by others[23].

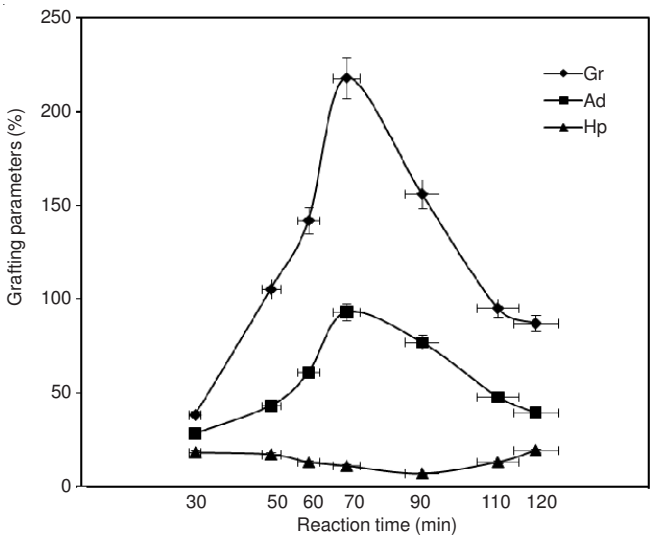


Fig. 7. Effect of the reaction time on the grafting parameters; Reaction conditions: Collagen 4 wt %, APS 0.04 mol L^{-1}, AMPS 0.8 mol L^{-1}, temperature 60 ºC

Conclusion

The monomer, 2-acrylamido-2-methylpropanesulfonic acid (AMPS), can be easily graft copolymerized onto collagen using ammonium persulfate (APS) as an initiator in aqueous medium. In order to prove that 2-acrylamido-2-methylpropanesulfonic acid molecules were grafted, FTIR spectroscopy, TGA analysis were used. The reaction conditions were attempted to optimize for obtaining graft copolymers with higher grafting parameters. So, the reaction conditions for achieving the maximum % Gr (218) and % Ad (93) were found to be as follows: APS 0.04 mol/L, AMPS 0.8 mol/L, collagen 4 wt%, reaction temperature 60 ºC and reaction time 70 min.

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